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Three novel species of *Distoseptispora* (Distoseptisporaceae) isolated from bamboo in Jiangxi Province, China

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Abstract

Decaying bamboo in freshwater is a unique eco-environment for fungi. Three new *Distoseptispora* (*Distoseptisporaceae*) species, *D. meilingensis*, *D. yongxiuensis* and *D. yunjushanensis* from submerged decaying bamboo culms in Jiangxi Province, China, were discovered, based on phylogenetic analyses and morphological characters. The combined data of ITS-LSU-SSU-*Tef*1 sequences were used to infer the phylogenetic relationship between *D. meilingensis*, *D. yongxiuensis*, *D. yunjushanensis* and related species. Both molecular analyses and morphological data supported *D. meilingensis*, *D. yongxiuensis* and *D. yunjushanensis* as three independent taxa.

Keywords

Hyphomycetes, phylogenetic analysis, *Sordariomycetes*, taxonomy, three new taxa

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Introduction

Distoseptispora was established by Su et al. (2016) as the single genus in *Distoseptisporaceae*. This genus morphologically resembles *Ellisembia* and *Sporidesmium* (Subramanian 1992; Shenoy et al. 2006; Yang et al. 2018), while they are not in sister clades in molecular phylogenetic trees (Su et al. 2016; Luo et al. 2019; Hyde et al. 2020, 2021). Multigene analysis showed that *Distoseptispora* formed a stable and well-supported clade within *Distoseptisporales* as a sister clade to *Aquapteridospora* (Luo et al. 2019; Hyde et al. 2020, 2021). *Aquapteridospora* has been raised as a new family *Aquapteridosporaceae* for the divergence time (110 million years ago (mya)) falling within the family-level range (50–130 mya) (Hyde et al. 2021). *Aquapteridospora* and *Distoseptispora* are similar in having macronematous, mononematous, unbranched conidiophores, mono- or polyblastic, holoblastic, conidiogenous cells and acrogenous, solitary conidia. *Distoseptispora* can easily be distinguished from *Aquapteridospora* by its short conidiophores and obclavate or cylindrical, rostrate, euseptate or distoseptate conidia. Additionally, *Distoseptispora* has terminal conidiogenous cells which lack circular scars (Hyde et al. 2021).

Distoseptispora was regarded as saprobic lignicolous fungal genus, which has the ability to decompose lignocelluloses in wood (Wong et al. 1998; Hyde et al. 2016). In recent years, the number of new taxa in *Distoseptispora* is steadily increasing and currently comprises 35 species, which have been discovered mostly in freshwater and some in terrestrial habitats (Su et al. 2016; Dong et al. 2021; Hyde et al. 2021; Li et al. 2021). Except for the two species, *D. adscendens* and *D. leonensis*, which were found from Hungary and Malaysia, respectively (Shoemaker and White 1985; Mckenzie 1995), 19 of the 33 species has been discovered in Thailand, while the remaining 14 species were introduced from China (Table 2). In China, *Distoseptispora* species are almost exclusively reported in Yunnan Province (Su et al. 2016; Luo et al. 2018; Hyde et al. 2019; Phookamsak et al. 2019; Li et al. 2021). Only three species, *D. martinii*, *D. bambusae* and *D. suoluoensis*, have been discovered from Guizhou Province (Xia et al. 2017; Yang et al. 2018; Sun et al. 2020). In this study, we introduce three new species of *Distoseptispora*, including *D. meilingensis*, *D. yongxiuensis* and *D. yunjushanensis* from Jiangxi Province in subtropical China. We describe the novel species, based on morphological illustrations and phylogenetic analyses. A synopsis of the morphological characters of *Distoseptispora* species is also provided.

Materials and methods

Samples collection, morphological observation and isolation

Dead bamboo samples from different freshwater habitats in Jiangxi Province, China, were taken to the lab for detection of fungi using a Nikon SMZ-1270 microscope (Nikon Corporation, Japan). Micro-morphological characteristics were observed and

captured using a Nikon ECLIPSE Ni-U compound microscope (Nikon Corporation, Japan), equipped with a Nikon DS-Fi3 camera. All measurements were calculated using PhotoRuler Ver. 1.1 software (The Genus Inocybe, Hyogo, Japan) and figures were processed using Adobe Photoshop CS6 Extended version 10.0 software (Adobe Systems, USA). Pure cultures of the fungi were obtained by the single spore isolation method (Chomnunti et al. 2014). The germinating conidia were transferred to potato dextrose agar (PDA) and incubated at 25 °C for two weeks. The fungal cultures were deposited in the Jiangxi Agricultural University Culture Collection (JAUCC) and the holotypic specimens with MycoBank numbers (842065, 842066, 842067) were deposited in the Herbarium of Fungi, Jiangxi Agricultural University (HFJAU) .

DNA extraction, PCR amplification and sequencing

Fungal genomes were extracted from fresh mycelium using a modified cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle 1987). Four deoxyribonucleic acid (DNA) barcodes (ITS, LSU, SSU and *Tef-1α*) were chosen for polymerase chain reaction (PCR) using the primer pairs ITS1/ITS4 (White et al. 1990), LR0R/LR7 (Hopple and Vilgalys 1999), NS1/NS4 (White et al. 1990) and EF983F/EF2218R (Örstadius et al. 2015), respectively. Amplification reactions were carried out in a volume of 25 μl, containing 12.5 μl 2 × Taq PCR MasterMix (Qingke, Changsha, China), 1 μl each forward and reverse primer (0.2 μM), 1 μl template DNA (circa 50–100 ng) and 9.5 μl ddH_2O. Amplifications were conducted under the following conditions: 3 min at 98 °C, 35 cycles of 10 s at 98 °C, 10 s of annealing at 55 °C and extension at 72 °C for 10 s, with a final 2-min extension at 72 °C. Sequencing reactions were conducted with the corresponding forward and reverse primers commercially by QingKe Biotechnology Co. (Changsha, China). All sequences were edited with Sequencher v.4.14 (GeneCodes Corporation, USA) and have been deposited in the NCBI GenBank database (Table 1).

Data analyses

Reference sequences of 35 *Distoseptispora* species and three *Aquapteridospora* species, based on recent publications (Luo et al. 2019; Hyde et al. 2020; Monkai et al. 2020; Dong et al. 2021, Li et al. 2021) were downloaded from GenBank. Detailed information on fungal strains used in this paper are provided in Table 1.

All obtained sequences were aligned using the online service of MAFFT (Madeira et al. 2019) and refined manually in MEGA v.7.0 (Kumar et al. 2016). Maximum Likelihood (ML) analysis was conducted with RAxML 8.0 using a GTR-GAMMA model of evolution (Stamatakis 2014). Non-parametric bootstrap analysis was implemented using 1,000 replicates to estimate ML bootstrap (BS) values. Bayesian Inference (BI) analysis was carried out with MrBayes v.3.2 under partitioned models (Ronquist et al. 2012). The best-fit models of nucleotide substitutions were selected according to the Akaike Information Criterion (AIC) implemented in jModelTest2.1.1

Table 1. Sequences used in this study.

Taxa	Voucher	LSU	ITS	SSU	*Tef-1α*
Aquapteridospora aquatica	MFLUCC 17-2371	NG_075413	NR_172447	—	—
Aquapteridospora fusiformis	MFLU 18-1601	MK849798	MK828652	—	MN194056
Aquapteridospora lignicola	MFLU 15-1172	KU221018	—	—	—
Distoseptispora adscendens	HKUCC 10820	DQ408561	—	—	—
Distoseptispora appendiculata	MFLUCC 18-0259	MN163023	MN163009	—	MN174866
Distoseptispora aquatica	GZCC 19-0452	MZ227216	MW133908	MW134689	—
Distoseptispora aquatica	MFLUCC 16-0904	MK849794	MK828649	MK828315	—
Distoseptispora aquatica	MFLUCC 18-0646	MK849793	MK828648	—	—
Distoseptispora aquatica	MFLUCC 16-1357	MK849796	MK828650	MK828317	—
Distoseptispora aquatica	S-965	MK849792	MK828647	MK828314	MN194051
Distoseptispora bambusae	MFLUCC 20-0091	NG_074430	NR_170068	NG_070348	—
Distoseptispora bambusae	MFLU 20-0261	MT232718	MT232713	MT232716	MT232880
Distoseptispora bambusae	MFLU 17-1653	MT232717	MT232712	—	—
Distoseptispora cangshanensis	MFLUCC 16-0970	MG979761	MG979754	—	MG988419
Distoseptispora caricis	CPC 36498	MN567632	NR_166325	—	—
Distoseptispora clematidis	MFLUCC 17-2145	MT214617	MT310661	MT226728	—
Distoseptispora clematidis	KUN-HKAS 112708	MW879523	MW723056	MW774580	—
Distoseptispora dehongensis	KUMCC 18-0090	MK079662	MK085061	—	MK087659
Distoseptispora euseptata	MFLUCC 20–0154	MW081544	MW081539	—	—
Distoseptispora euseptata	DLUCC S2024	MW081545	MW081540	—	MW084994
Distoseptispora fasciculata	KUMCC 19-0081	NG_075417	NR_172452	—	MW396656
Distoseptispora fluminicola	DLUCC 0391	MG979762	MG979755	—	MG988420
Distoseptispora fluminicola	DLUCC 0999	MG979763	MG979756	—	MG988421
Distoseptispora guttulata	MFLU 17-0852	MF077554	MF077543	MF077532	MF135651
Distoseptispora hydei	MFLUCC 20-0481	MT742830	MT734661	—	—
Distoseptispora leonensis	HKUCC 10822	DQ408566	—	—	—
Distoseptispora lignicola	MFLUCC 18-0198	MK849797	MK828651	MK828318	—
Distoseptispora longispora	HFJAU 0705	MH555357	MH555359	MH555431	—
Distoseptispora martinii	CGMCC 318651	KX033566	KU999975	KX033537	—
Distoseptispora meilingensis	**JAUCC 4727**	**OK562396**	**OK562390**	**OK562402**	**OK562408**
Distoseptispora meilingensis	**JAUCC 4728**	**OK562397**	**OK562391**	**OK562403**	**OK562409**
Distoseptispora multiseptata	MFLUCC 15-0609	KX710140	KX710145	NG_065693	MF135659
Distoseptispora multiseptata	MFLU 17-0856	MF077555	MF077544	MF077533	—
Distoseptispora neorostrata	MFLUCC 18-0376	MN163017	MN163008	—	—
Distoseptispora obclavata	MFLUCC 18-0329	MN163010	MN163012	—	—
Distoseptispora obpyriformis	DLUCC 0867	MG979765	MG979757	—	MG988423
Distoseptispora palmarum	MFLUCC 18-1446	MK079663	MK085062	MK079661	MK087660
Distoseptispora palmarum	MFLU 18-0588	NG_067856	NR_165897	—	MK087660
Distoseptispora phangngaensis	MFLU 17-0855	MF077556	MF077545	MF077534	MF135653
Distoseptispora phangngaensis	MFLUCC 16-0857	—	NR_166230	—	—
Distoseptispora rayongensis	MFLUCC 18-0415	NG_073624	NR_171938	NG_073504	—
Distoseptispora rayongensis	MFLU 18-1045	MH457137	MH457172	MH457169	—
Distoseptispora rostrata	MFLUCC 16-0969	MG979766	MG979758	—	MG988424
Distoseptispora rostrata	DLUCC 0885	MG979767	MG979759	—	MG988425
Distoseptispora rostrata	MFLU 18-0479	NG_064513	NR_157552	—	—
Distoseptispora saprophytica	MFLUCC 18-1238	NG_075419	NR_172454	—	MW396651
Distoseptispora songkhlata	MFLUCC 18-1234	MW287755	MW286482	—	MW396642
Distoseptispora submersa	MFLUCC 16-0946	MG979768	MG979760	—	MG988426
Distoseptispora suoluoensis	MFLUCC 17-0224	NG_068552	NR_168764	NG_070113	MF135654
Distoseptispora suoluoensis	MFLU 17-0854	MF077558	MF077547	MF077536	—
Distoseptispora tectonae	MFLUCC 15-0981	MW287763	MW286489	—	MW396641
Distoseptispora tectonae	MFLUCC 12-0291	KX751713	KX751711	—	KX751710

Taxa	Voucher	LSU	ITS	SSU	*Tef-1α*
Distoseptispora tectonae	S-2023	MW081543	MW081538	—	—
Distoseptispora tectonae	GZ 25	MH555358	MH555361	—	—
Distoseptispora tectonigena	MFLUCC 12-0292	KX751714	NR_154018	—	—
Distoseptispora thailandica	MFLUCC 16-0270	MH260292	MH275060	MH260334	MH412767
Distoseptispora thysanolaenae	KUN-HKAS 112710	MW879524	MW723057	—	—
Distoseptispora thysanolaenae	KUN-HKAS 102247	MK064091	MK045851	—	MK086031
Distoseptispora xishuangbannaensis	KUMCC 17-0290	MH260293	MH275061	MH260335	MH412768
Distoseptispora yongxiuensis	**JAUCC 4725**	**OK562394**	**OK562388**	**OK562400**	**OK562406**
Distoseptispora yongxiuensis	**JAUCC 4726**	**OK562395**	**OK562389**	**OK562401**	**OK562407**
Distoseptispora yunjushanensis	**JAUCC 4723**	**OK562398**	**OK562392**	**OK562404**	**OK562410**
Distoseptispora yunjushanensis	**JAUCC 4724**	**OK562399**	**OK562393**	**OK562405**	**OK562411**
Distoseptispora yunnansis	MFLUCC 20–0153	MW081546	MW081541	—	MW084995

"—", sequence is unavailable.

(Darriba et al. 2012) on XSEDE in the CIPRES web portal (Miller et al. 2010). The models for ITS, LSU, SSU and *Tef-1α* datasets used for phylogenetic analysis are GTR+I+G model (-lnL = 4965.1122), GTR+I+G model (-lnL = 2716.7536), TIM2+G (-lnL = 4344.2295) and TrN+I+G (-lnL = 4479.4914), respectively. The datasets were run for 10,000,000 generations, with four chains and trees sampled every 1,000 generations. The first 10% trees were discarded as burn-in. We used three *Aquapteridospora* species as outgroups. The Bayesian consensus tree with posterior probabilities (PP) was visualised with FigTree v.1.4.4 (Rambaut 2018) and was edited in Adobe Illustrator CS6. Our aligned matrices and trees can be obtained from TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S29465).

Results

Molecular phylogenetic results

According to the results of BLAST analysis and sequence alignment, the ITS sequence of *D. meilingensis* has 11 different loci from those of *D. yongxiuensis*, the ITS sequence of which shares 99% similarity (five different loci) with that of *D. suoluoensis*. The ITS sequence of *D. yunjushanensis* is 97% similar (22 different loci) to that of *D. obclavata*. The aligned matrix for the combined analysis, ITS + LSU + SSU + *Tef-1α*, had 4015 bp, including ITS 596 bp, LSU 799 bp, SSU 1715 bp and *Tef-1α* 905 bp. The topologies of trees generated by ML and BI analyses are highly similar. The Bayesian tree with BS and PP is shown in Fig. 1. All species of *Distoseptispora* form a monophyletic group (BS/PP = 100/1.00). *D. yongxiuensis* groups together with *D. suoluoensis* (BS/PP = 60/0.99). These two species and collections of *D. meilingensis* form a strong-supported clade (BS/PP = 99/1.00), which is strongly linked with sequences of *D. bambusae* (BS/PP = 100/1.00). Collections of *D. yunjushanensis* form a moderate-support clade (BS/PP = 81/1.00) with the lineage consisting of *D. obclavata* and *D. rayongensis*.

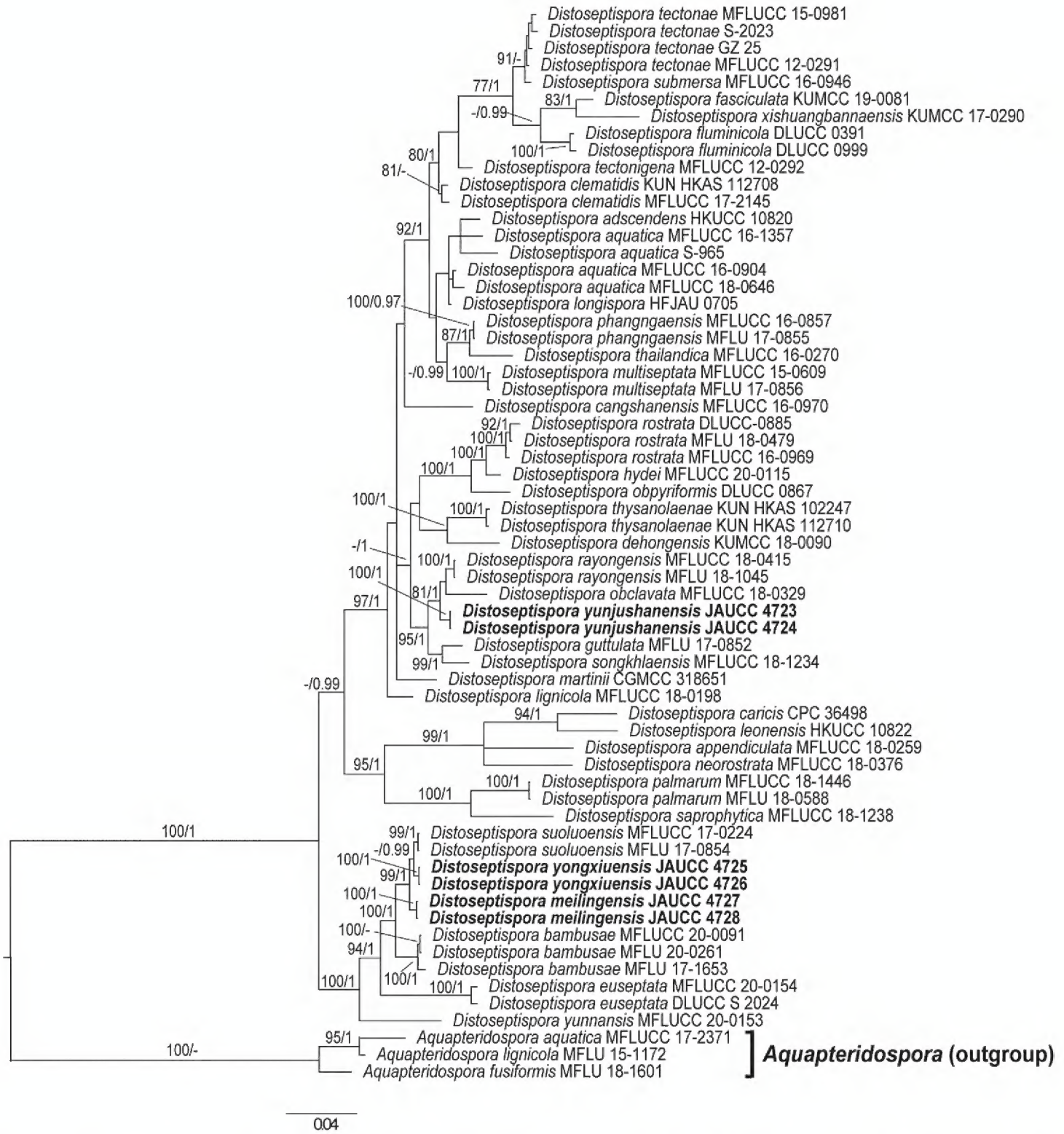

Figure 1. Phylogenetic tree of *Distoseptispora*, inferred from the combined regions (ITS-LSU-SSU-*Tef 1α*) using Bayesian Inference (BI) analysis. The *Aquapteridospora* clade was used as the outgroup. The lineages with new species were shown in bold. PP ≥ 0.95 and BS ≥ 75% were indicated around the branches. The new sequences generated in this study are given in bold.

Taxonomy

***Distoseptispora meilingensis* Z. J. Zhai & D. M. Hu, sp. nov.**

MycoBank No: 842067

Fig. 2

Etymology. Referring to the collecting site of the Meiling Mountain in Jiangxi Province, China.

Holotype. HFJAU 10009.

Figure 2. *Distoseptispora meilingensis* (HFJAU10009, holotype) **a, b** colonies on bamboo culms **c–e** conidiophores with conidia **f** conidiogenous cells **g, n** conidiogenous cells with conidia **h–m** conidia **o** germinating conidium **p** culture on PDA from above and reverse. Scale bars: 100 μm (**a, b**), 20 μm (**c–e, o**), 5 μm (**f-n**).

Description. Saprobic on culms of bamboo. ***Sexual morph*:** Undetermined. ***Asexual morph*:** Hyphomycetous. ***Colonies*** effuse, brown to dark brown, hairy. *Mycelium* mostly immersed, composed of pale to dark brown, septate, branched, smooth, hyaline to subhyaline hyphae. ***Conidiophores*** 69–192 × 4–7 μm ($\bar{x}$ = 120.6 × 5.5 μm, n = 25), macronematous, mononematous, erect, cylindrical, straight or slightly flexuous, 5–12-septate, yellowish-brown or brown, robust at the base. ***Conidiogenous cells*** holoblastic, mono- to polyblastic, integrated, terminal, cylindrical, yellowish-brown or brown. ***Conidia*** 32–64.5 × (7–)9–12.5 μm ($\bar{x}$ = 43.7 × 9.8 μm, n = 30), acrogenous, solitary, straight or slightly curved, obclavate, 5–7-distoseptate, thick-walled, rounded at the apex, truncate at the base, tapering towards apex, bud scars disjunctors at base, mostly brown when mature.

Cultural characteristics. Conidia germinating on PDA within 24 h and germ tubes produced from both ends. Colonies on PDA reaching 17–23 mm diam. at two weeks at 25 °C, in natural light, circular, with dense, light olivaceous mycelium on the surface with entire margin; reverse brown to dark brown.

Material examine. China, Jiangxi Province, Nanchang City, Meiling Mountain, alt. 305 m, near 28.79°N, 115.72°E, on decaying bamboo culms submerged in a freshwater stream, 16 Aug 2021, Z. J. Zhai, SLT-3 (HFJAU10009, ***holotype***), ex-type living culture, JAUCC 4727 = JAUCC 4728.

Notes. *Distoseptispora meilingensis* clusters with the clade including *D. suoluoensis* and *D. yongxiuensis* with high support in the phylogenetic tree (Fig. 1). *Distoseptispora meilingensis* is distinct from *D. suoluoensis* (Yang et al. 2018) and *D. yongxiuensis* by its conidial colour (mostly brown, yellowish-brown to dark olivaceous and yellowish-brown or brown, respectively). Furthermore, *D. meilingensis* has shorter conidia (32–64.5 μm vs. (65–)80–125(–145) μm) than those of *D. suoluoensis* (Yang et al. 2018) and slightly shorter conidiophores (69–192 μm vs. 112–253 μm) than those of *D. yongxiuensis*. *Distoseptispora meilingensis* resembles *D. bambusae* in similar habitats and polyblastic conidiogenous cells (Sun et al. 2020). However, *D. meilingensis* can be distinguished from *D. bambusae* in its longer conidiophores (69–192 μm vs. 40–96 μm), slightly wider (up to 12.5 μm vs. up to 9.5 μm) and brighter (light brown vs. brown) conidia (Sun et al. 2020). A comparison of morphological features of *Distoseptispora* species is provided in Table 2.

***Distoseptispora yongxiuensis* Z. J. Zhai & D. M. Hu, sp. nov.**
MycoBank No: 842066
Fig. 3

Etymology. With reference to Yongxiu, from where the holotype was collected.

Holotype. HFJAU10007

Description. Saprobic on decaying bamboo culms. ***Sexual morph*:** Undetermined. ***Asexual morph*:** Hyphomycetous. ***Colonies*** effuse, brown, hairy, glistening, often inconspicuous. ***Mycelium*** partly superficial, partly immersed in the substra-

Table 2. Synopsis of morphological characteristics, habitats, hosts and district compared across *Distoseptispora* species.

Species	Conidiophores (μm)	Conidia (μm)	Conidia septation	Conidia characteristics	Habitat	Host	District	References
Distoseptispora meilingensis	**69–192 × 4–7**	**32–64.5 × (7–)9–12.5**	**5–7-distoseptate**	**Obclavate, mostly bright brown when mature**	**Freshwater**	**Dead bamboo culms**	**China, Jiangxi**	**This study**
D. yongxiuensis	**112–253 × 4–9**	**46–74(–86) × 10–13(–16)**	**6–9-euseptate**	**Obclavate or obspathulate, olivaceous to yellowish-brown or brown, guttulate**	**Freshwater**	**Dead bamboo culms**	**China, Jiangxi**	**This study**
D. yunjushanensis	**100–175 × 5.5–10**	**39–67.5(–77) × (7–)9.5–13.5(–16.5)**	**7–13-distoseptate**	**Obpyriform or obclavate, olivaceous when young, dark brown when mature**	**Freshwater**	**Dead bamboo culms**	**China, Jiangxi**	**This study**
D. adscendens	28–46 × 8–10	(80–)350–500 × 15–18	80-distoseptate	Cylindrical, hemispherical apex, hyaline	Terrestrial	Decaying wood of *Fagus sylvatica*	Hungary	Shoemaker and White (1985), Réblová (1999)
D. appendiculata	62–86 × 4.5–5.5	67–89 × 10–16	13–17-distoseptate	Obpyriform or obclavate, olivaceous or dark brown, with gelatinous sheath around tip	Freshwater	Unidentified submerged wood	Thailand, Khwaeng Phra	Luo et al. (2019)
D. aquatica	29–41 × 7–9	110–157 × 13.5–16.5	15–28-distoseptate	Obclavate, dark brown with bluish-green to malachite green tinge	Freshwater	Unidentified submerged wood	China, Yunnan	Su et al. (2016)
D.bambusae	40–96 × 4–5.5	45–74 × 5.5–9.5	5–10-distoseptate	Obclavate, olivaceous or brown	Terrestrial	Dead bamboo culms	China and Thailand	Sun et al. (2020), Monkai et al. (2020)
D. cangshanensis	44–68 × 4–8	58–166(–287) × 10–14	Multi-distoseptate	Obclavate or lanceolate, rostrate, olivaceous or brown	Freshwater	Unidentified submerged wood	China, Yunnan	Luo et al. (2018)
D. caricis	35–90 × 6–7	(55–)65–85(–100) × 15–16(–17)	5–10-distoseptate	Obclavate, brown, septa with central pore, basal cell pale brown, with truncate hilum	Terrestrial	Leaves of *Carex* sp.	Thailand, Chiang Mai	Crous et al. (2019)
D. clematidis	22–40 × 4–10	120–210 × 12–20	28–35-distoseptate	Oblong, obclavate, cylindrical or rostrate, brown with green tinge, bud scars or disjunctors present at the site of attachment	Terrestrial	Dried branches of *Clematis sikkimensis*	Thailand, Chiang Rai	Phukhamsakda et al. (2020)
D. dehongensis	45–80 × 4–5	17–30 × 7.5–10	3–5-distoseptate	Obpyriform to obclavate, broad cylindrical or irregular, olivaceous	Freshwater	Unidentified submerged wood	China, Yunnan	Hyde et al. (2019)
D. euseptata	19–28 × 4–5	37–54 × 8–9	4–7-euseptate	Obpyriform to obclavate, often constricted at septa, olivaceous	Freshwater	Unidentified submerged wood	China, Yunnan	Li et al. (2021)

Species	Conidiophores (μm)	Conidia (μm)	Conidia septation	Conidia characteristics	Habitat	Host	District	References
D. fasciculata	12–16 × 5–6	46–200 × 10–16.5	10–40-distoseptate	Subcylindrical to obclavate, olivaceous when young, dark brown when mature	Freshwater	Unidentified submerged wood	Thailand, Nakhon Si Thammarat	Dong et al. (2021)
D. fluminicola	21–33 × 5.5–6.5	125–250 × 13–15	17–34-distoseptate	Oblong, obclavate, cylindrical or rostrate, brown with green tinge	Freshwater	Unidentified submerged wood	China, Yunnan	Su et al. (2016)
D. guttulata	55–90(–145) × 3.5–5.5	75–130(–165) × 7–11	11–14(–20)-euseptate	Obclavate or lanceolate, rostrate, mid to dark brown or olivaceous	Freshwater	Unidentified submerged wood	Thailand, Prachuap Khiri Khan	Yang et al. (2018)
D. hydei	87–145 × 3–7	32–58 × 10–15	7–9-distoseptate	Obpyriform to fusiform, olivaceous to brown, with a hyaline, globose, gelatinous sheath around tip	Terrestrial	Dead bamboo culms	Thailand, Phitsanulok	Monkai et al. (2020)
D. leonensis	Up to 175 × 6–7	(38–)50–75(–85) × 11–15	7–12-distoseptate	Obclavate, rostrate, brown	Terrestrial	Dead culms of *Freycinetia* sp.	Malaysia	McKenzie (1995)
D. lignicola	84–124 × 4–5	60–108 × 7–9	5–9-euseptate	Obclavate, curved, brown	Freshwater	Unidentified submerged wood	Thailand, Sai-Khu Waterfall	Luo et al. (2019)
D. longispora	17–37 × 6–10	189–297 × 16–23	31–56-distoseptate	Obclavate, elongated, brown to yellowish-brown	Freshwater	Unidentified submerged wood	China, Yunnan	Song et al. (2020)
D. martinii	50–110 × 3.5–4.5	15–20 × 11–16	Transversal septa	Transversal ellipsoid, oblate or subglobose, muriform, pale brown to brown	Terrestrial	Unidentified dead branches	China, Guizhou	Xia et al. (2017)
D. multiseptata	29–47 × 4–6	147–185 × 12–14	Multi-distoseptate	Obclavate, rostrate, dark olivaceous green	Freshwater	Unidentified submerged wood	Thailand, Prachuap Khiri Khan	Hyde et al. (2016)
D. neorostrata	93–117 × 5.5–6.5	109–147 × 13–15	Multi-distoseptate	Obclavate, rostrate, dark olivaceous to mid or dark brown	Freshwater	Unidentified submerged wood	Thailand, Khwaeng Phra Khanong Nuea	Luo et al. (2019)
D. obclavata	117.5–162.5 × 5–7	46–66 × 9–11	9-11-distoseptate	Obclavate, olivaceous to pale or dark brown, guttulate	Freshwater	Unidentified submerged wood	Thailand, Khwaeng Phra Khanong Nuea	Luo et al. (2019)
D. obpyriformis	97–119 × 5–7	53–71 × 12–16	9–11-distoseptate	Obpyriform, olivaceous to pale or dark brown, guttulate	Freshwater	Unidentified submerged wood	China, Yunnan	Luo et al. (2018)
D. palmarum	90–165 × 4–7	35–180 × 7–11	7–27-distoseptate	Oblong, obclavate, greenish-black to brown	Terrestrial	Rachis of *Cocos nucifera*	Thailand, Trat	Hyde et al. (2019)
D. phangngaensis	18–30(–40) × 4.3–6.5	165–350 × 14–19	Multi-distoseptate	Elongate, obclavate, rostrate, dark olivaceous to mid or dark brown	Freshwater	Unidentified submerged wood	Thailand, Phang Nga	Yang et al. (2018)

Species	Conidiophores (μm)	Conidia (μm)	Conidia septation	Conidia characteristics	Habitat	Host	District	References
D. rayongensis	75–125 × 3.5–5.5	(36–)60–106(–120) × 9–14.5	9–13-euseptate, rarely 14–15-septate	Obclavate or obspathulate, rostrate, pale brown or pale olivaceous, with percurrent proliferation	Freshwater	Unidentified submerged wood	Thailand, Rayong	Hyde et al. (2020)
D. rostrata	82–126 × 5–7	115–155 × 9–11	(15–)18–23-distoseptate	Obclavate or lanceolate, rostrate, olivaceous to pale brown	Freshwater	Unidentified submerged wood	China, Yunnan	Luo et al. (2018)
D. saprophytica	50–140 × 3.2–4.2	14.5–30 × 4.5–7.5	2–6-distoseptate	Subcylindrical to obclavate, olivaceous to brown	Freshwater	Unidentified submerged wood	Thailand, Songkhla	Dong et al. (2021)
D. songkhlaensis	70–90 × 4–5.5	44–125 × 9–14.5	9–16-distoseptate	Obclavate, constricted at septa, olivaceous to brown	Freshwater	Unidentified submerged wood	Thailand, Songkhla	Dong et al. (2021)
D. submersa	55–73 × 7–9	95–123 × 15–19	17–23(–28)-distoseptate	Obclavate, brown to dark brown or olivaceous	Freshwater	Unidentified submerged wood	China, Yunnan	Luo et al. (2018)
D. suoluoensis	80–250 × 4.5–5.8	(65–)80–125(–145) × 8–13	8–10-euseptate	Narrowly obclavate or obspathulate, yellowish-brown or dark olivaceous, verrucose, with percurrent proliferation	Freshwater	Unidentified submerged wood	China, Guizhou	Yang et al. (2018)
D. tectonae	19.5–95 × 4.5–9	45–270 × 11–16	10–40-distoseptate	Obclavate, brown to dark brown or olivaceous	Terrestrial/ Freshwater	Dead twig of *tectona grandis* (Lamiaceae)	Thailand, Prachuap Khiri Khan	Hyde et al. (2016)
D. tectonigena	Up to 110 × 5–11	(83–)148–225(360–) × (10–)11–12(–13)	20–46-distoseptate	Flexuous, cylindrical-obclavate, elongated, verruculose, dark reddish-brown	Terrestrial	Dead twig of *Tectona grandis* (Lamiaceae)	Thailand, Chiang Rai	Hyde et al. (2016)
D. thailandica	15–26 × 3–6	130–230 × 13.5–17	35–52-distoseptate	Oblong, obclavate, cylindrical or rostrate, reddish-brown to brown	Terrestrial	Dead leaves of *Pandanus* sp.	Thailand, Prachuap Khiri Khan	Tibpromma et al. (2018)
D. thysanolaenae	30–80 × 3.5–5.5	21.5–80 × 6.5–12.8	8–14-distoseptate	Elongated obclavate, light to dark brown, flat apex, with conspicuous spore attachment loci	Terrestrial	Dead culms of *Thysanolaena maxima*	China, Yunnan	Phookamsak et al. (2019)
D. xishuangbannaensis	12–17 × 2–5	160–305 × 8–15	Up to 40-distoseptate	Cylindrical-obclavate, green-brown to brown, tapering towards apex	Terrestrial	Dead leaf sheaths of *Pandanus utilis*	China, Yunnan	Tibpromma et al. (2018)
D. yunnanensis	131–175 × 6–7	58–108 × 8–10	6–10-euseptate	Obclavate, rostrate, mid-olivaceous to brown	Freshwater	Unidentified submerged wood	China, Yunnan	Li et al. (2021)

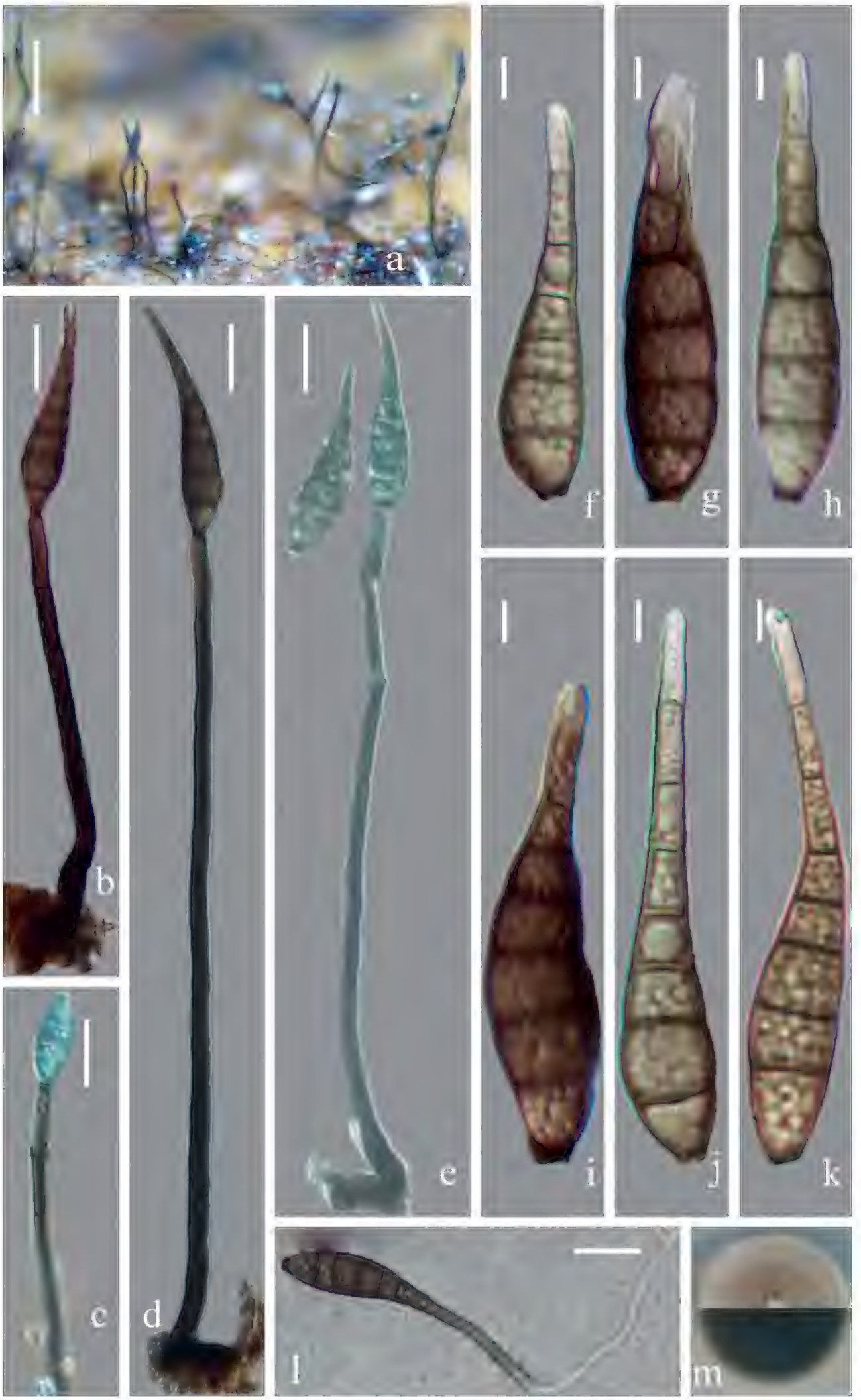

Figure 3. *Distoseptispora yongxiuensis* HFJAU 10007, holotype) **a** Colonies on bamboo culm **b, d** conidiophores with conidia **c** conidiogenous cell bearing conidium **e** conidiogenous cells with young conidia **f-k** conidia **l** germinating conidium **m** culture on PDA from above and reverse. Scale bars: 100 μm (**a**), 20 μm (**b–e, l**), 5 μm (**f–k**).

tum, composed of hyaline to pale brown, septate, branched hyphae. ***Conidiophores*** 112–253 × 4–9 μm ($\bar{x}$ = 198 × 6.9 μm, n = 15), macronematous, mononematous, solitary or aggregated at the base, cylindrical, straight or slightly flexuous, 8–13-septate, olivaceous to dark brown, sharply curving near the base, paler at the apical part, rounded at the apex. ***Conidiogenous cells*** integrated, terminal, monoblastic, rarely polyblastic, cylindrical, olivaceous to dark brown. ***Conidia*** 46–74(−86) × 10–13(−16) μm ($\bar{x}$ = 65.6 × 12.6 μm, n = 30), acrogenous, solitary, obclavate or obspathulate, straight or flexuous, rostrate, 6–9-euseptate, olivaceous to yellowish-brown or brown, becoming paler or hyaline towards the apex, guttulate, 2.5–4 μm wide at the base and 2.5–5 μm wide at the apex, with a darkened scar at the base.

Cultural characteristics. Conidia germinating on PDA within 24 h and germ tubes produced from both ends. Colonies on PDA reaching 24–32 mm diam. at two weeks at 25 °C, in natural light, circular, with dense, light olivaceous mycelium on the surface with entire margin; reverse dark brown to black.

Material examined. China, Jiangxi Province, Jiujiang City, Yongxiu County, alt. 680.5 m, 29.09°N, 115.62°E, on decaying bamboo culms submerged in a freshwater stream, 28 Apr 2020, Z. J. Zhai and W. W. Li, YJS-70 (HFJAU 10007, ***holotype***), ex-type living culture, JAUCC 4725 = JAUCC 4726.

Notes. In the multi-gene phylogenetic tree (Fig. 1), *D. yongxiuensis* clusters with *D. suoluoensis*. Nonetheless, *D. yongxiuensis* can be distinguished from *D. suoluoensis* by its shorter conidia (46–74(−86) μm vs. (65–)80–125(–145) μm) and polyblastic conidiogenous cells (Yang et al. 2018). Additionally, *D. suoluoensis* has the percurrent proliferation of conidia, while it was not observed in *D. yongxiuensis*. *Distoseptispora yongxiuensis* is similar with *D. bambusae* (Sun et al. 2020), *D. palmarum* (Hyde et al. 2019) and *D. meilingensis* for the polyblastic conidiogenous cells, but *D. yongxiuensis* has wider conidia than those of *D. bambusae* (10–13(−16) μm vs. 5.5–9.5 μm) (Sun et al. 2020), shorter conidia than those of *D. palmarum* (46–74(−86) μm vs. 35–180 μm) (Hyde et al. 2019) and paler (yellowish-brown or brown vs. bright brown) conidia than those of *D. meilingensis*.

***Distoseptispora yunjushanensis* Z. J. Zhai & D. M. Hu, sp. nov.**
MycoBank No: 842065
Fig. 4

Etymology. The epithet refers to the collecting site from the Yunjushan Mountain in China.

Holotype. HFJAU10005

Description. Saprobic on decaying bamboo culms submerged in freshwater habitats. ***Sexual morph*:** Undetermined. ***Asexual morph*:** Hyphomycetous. ***Colonies*** effuse, olivaceous or dark brown, hairy, velvety. ***Mycelium*** mostly immersed, consisting of branched, septate, smooth, subhyaline to pale brown hyphae. ***Conidiophores*** 100–175 μm × 5.5–10 μm ($\bar{x}$ = 129×7.1 μm, n = 30), single or in groups of 2 or 3, macronematous, mononematous, erect, straight or slightly flexuous, 4–7-septate,

Figure 4. *Distoseptispora yunjushanensis* (HFJAU 10005, holotype) **a, b** colonies on bamboo culms **c–f** conidiophores with conidia **g-i** young conidia **j-l** mature conidia **m** conidium with proliferation **n** germinating conidium **o, p** culture on PDA from above and reverse. Scale bars: 100 μm (**a, b**), 20 μm (**c–f, m, n**), 5 μm (**g–l**).

unbranched, olivaceous to dark brown, smooth, cylindrical, rounded at the apex. ***Conidiogenous cells*** monoblastic, integrated, terminal, determinate, pale to dark brown, cylindrical. ***Conidia*** 39–67.5(–77) µm × (7–)9.5–13.5(–16.5) µm ($\bar{x}$ = 52 × 12 µm, n = 30), acrogenous, solitary, obpyriform or obclavate, thick-walled, tapering towards the rounded apex, slightly curved, truncate at the base, 7–13-distoseptate, guttulate, smooth-walled, olivaceous, dark brown when mature, sometimes with the percurrent proliferation which forms another conidium from the conidial apex.

Cultural characteristics. Conidia germinating on PDA within 24 h and germ tubes produced from both ends. Colonies on PDA reaching 12–18 mm diam. at 14 days at 25 °C, in natural light, with fluffy, dense, thin olivaceous mycelium in the centre, becoming sparse and paler at the entire margin; reverse dark brown, pale brown at the smooth margin.

Material examined. China, Jiangxi Province, Jiujiang City, Yongxiu County, Yunjushan Mountain, alt. 672.5 m, 29.23°N, 115.59°E, on decaying bamboo culms submerged in a freshwater stream, 28 Apr 2020, Z. J. Zhai and W. W. Li, YJS-42 (HFJAU 10005, ***holotype***), ex-type living culture, JAUCC 4723 = JAUCC 4724.

Notes. In the phylogenetic analysis, *D. yunjushanensis* clusters with *D. obclavata* and *D. rayongensis* with moderate support (BS/PP = 81/1.00). However, *D. yunjushanensis* is easily distinguished from *D. obclavata* by its comparatively wider (5.5–10 µm vs. 5–7 µm) conidiophores and conidia ((7–)9.5–13.5(–16.5) µm vs. 9–11 µm) (Luo et al. 2019). Moreover, the percurrent proliferation of conidia was not observed in *D. obclavata* (Luo et al. 2019). *Distoseptispora yunjushanensis* has shorter conidia (39–67.5(–77) µm vs. (36–)60–106(–120) µm) and wider conidiophores (5.5–10 µm vs. 3.5–5.5 µm) than those of *D. rayongensis* (Hyde et al. 2020). The morphology of *D. yunjushanensis* is similar to *D. guttulata* and *D. songkhlaensis* in having the obclavate conidia, but differs in having wider (5.5–10 µm vs. 3.5–5.5 µm and 4–5.5 µm) conidiophores, shorter (39–67.5(–77) µm vs. 75–130(–165) µm and 44–125 µm) and proliferating conidia (Yang et al. 2018; Dong et al. 2021). Additionally, *D. yunjushanensis* can be distinguished from *D. guttulata* by its distoseptate conidia (Yang et al. 2018).

Discussion

Previous reports of *Distoseptispora* were mainly concentrated in tropical areas, such as Thailand (Chiang Rai, Phitsanulok, Phang Nga; Luo et al. 2019) and southwest Yunnan, China (Su et al. 2016; Luo et al. 2018). Nonetheless, several new taxa were found sporadically in subtropical China, for example, *Distoseptispora martinii* (Xia et al. 2017), *D. suoluoensis* (Yang et al. 2018) and *D. bambusae* (Sun et al. 2020) in Guizhou Province and *D. euseptata* and *D. yunnansis* in northwest Yunnan (Li et al. 2021). The ongoing discovery of this taxa from other geographic regions in subtropical China will deepen our understanding of the species in this genus. In this study, we introduced another three new species of *Distoseptispora* from Jiangxi Province of subtropical China. It is interesting to note that all these species in subtropical China, except *D. yunjushanensis* and

D. martinii, formed a well-supported monophyletic clade in the phylogenetic tree and this clade was at the basal position (Fig. 1). *Distoseptispora yunjushanensis* and *D.martinii* were otherwise phylogenetically placed within other clades (Fig. 1) and, therefore, we suppose that other lineages might also comprise more *Distoseptispora* species distributed in subtropical China. Further discovery of *Distoseptispora* species in more extensive areas in subtropical and other regions of China are needed to be addressed if the phylogenetic position of species reflects their geographical and ecological distribution.

Distoseptisporaceae is a holomorphic group of *Sordariomycetes* that are saprobic on decaying wood and plant debris in terrestrial and freshwater habitats (Su et al. 2016). The genus *Distoseptispora* seems not to have specific habitat preferences, as most species were reported from submerged wood in freshwater habitats, while some were introduced from terrestrial habitats (Table 2). So far, only five species of *Distoseptispora* have been found on bamboo, two of them (*Distoseptispora bambusae* and *D. hydei*, Table 2) from terrestrial habitats, the other three (this study) from freshwater. There may be more species in this genus existing on bamboo waiting to be discovered and further studies are needed to clarify if a specific species in *Distoseptispora* is specific to its host.

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